Cell Lineage Analysis of Blastomeres of the Marginal Zone in the 32-Cell Stage Embryo of the Urodele Amphibian *Pleurodeles waltl*

Michel Delarue and J.C. Boucaut

Laboratoire de Biologie Expérimentale
URA CNRS 1135
Université P. et M. Curie
9, quai Saint-Bernard
75005 Paris, France

Abstract

Cell lineages of the blastomeres of the marginal zone in the 32-cell stage of *Pleurodeles waltl* embryo were analysed by intracytoplasmic injections of rhodamine-lysinated-dextran. The descendant cells of blastomeres contributed to more than one primary germ layer. The results also clearly demonstrate that slow mixing and rearrangement of cells are important mechanisms in the early steps of amphibian embryonic development.

Abridged English Version

The amphibian embryo is an attractive system to study gene expression in the first steps of development. Injection into one blastomere with antisense oligodeoxyribo-nucleotides to specifically inhibit maternal mRNAs or with *in vitro* transcribed mRNAs to deregulate normal expression are powerful stategies [[3], [4], [5], [6]). Disruption of protein function during the early cleavage period with cytoplasmic microinjection of antibodies has also been reported, both in *Xenopus* and in *Pleurodeles waltl* ([9], [10]). These technologies require knowledge of the fate of injected blastomeres during embryonic development. This may be achieved by the microinjection of a cell lineage tracer. Using a fluorescent dextran we have studied the fate of blastomeres at the 32-cell stage in the urodele *Pleurodeles waltl*. Since they are related to the future marginal zone and the formation of the mesoderm, we have focused our observations on blastomeres B and C [13] (Figure 1).

Intracellular injection was performed with selected embryos from one batch. Dorsal localization of injected B blastomeres was assessed using vital dye labelling. Individual blastomeres were injected with 10 nl of Rhodamine-Lysine-Dextran (RLDx) in 10% Normal Amphibian Medium (NAM) [14]. Injected embryos were allowed to develop up to the tail-bud stage and processed for histology. Sections (10 µm) were observed under epifluorescence.

Dorsal blastomeres. We have followed the fate of blastomeres B and C throughout development. As can be seen in Figure 2a, the progeny of blastomere B1 were found in the truncal and posterior parts of the embryo. They mainly formed the notochord, and dorsal parts of the sclerotome and myotome. B1 derived cells were present in the head mesen-

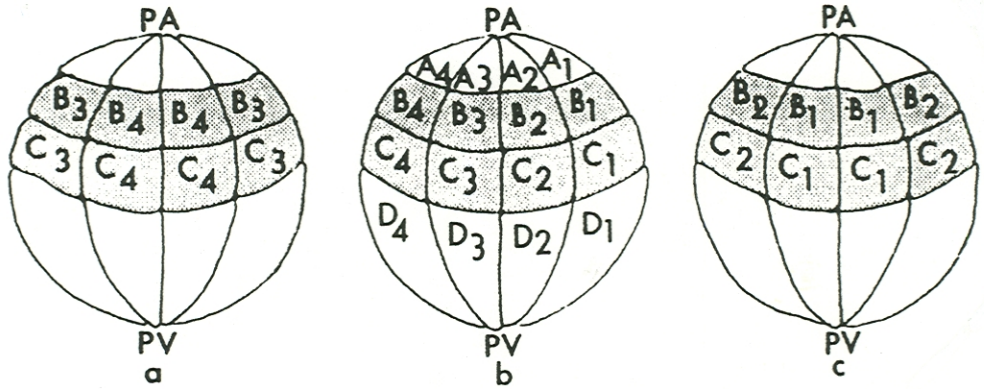
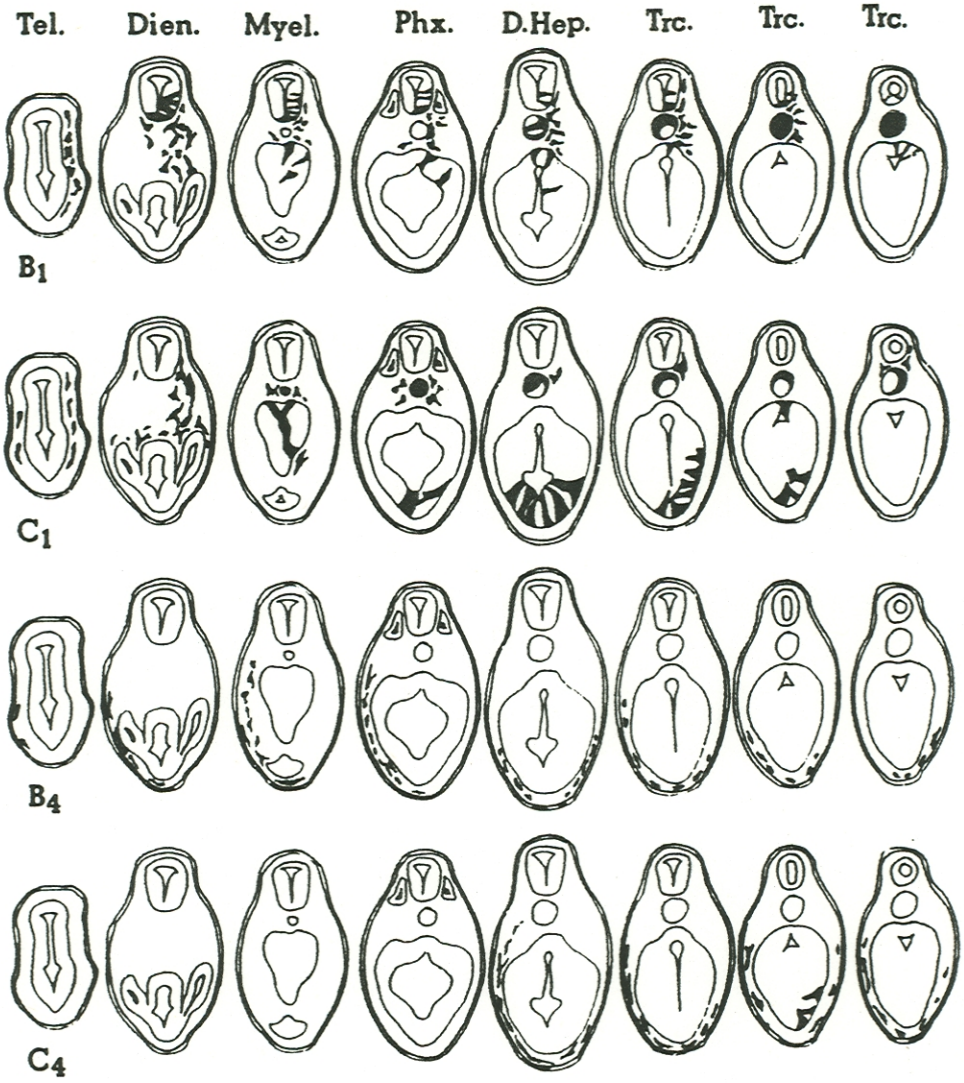


Figure 1. Schematic drawing of the 32-cell stage *Pleurodeles waltl* embryo. The blastomeres which fate had been analyzed are shaded. a) ventral view; b) lateral view; c) dorsal view. Nomenclature of blastomeres at the 32-cell stage is that previously defined for *Xenopus laevis* [13]. PA. Animal pole; PV. Vegetal pole.

Figure 2. Schematic representation of the fate of B1, C1, B4 and C4 injected blastomeres in embryos at the tail-bud stage. Transverse section levels are indicated as following : Tel. Telencephalon; Dien. Diencephalon; Myel. Myelencephalon; Phx. Pharynx; D. Hep. Liver diverticulum; Trc. Truncal.



chyme close to the otic vesicle, and in caudal mesoderm. Cells of the lateral and ventral regions of the rhombencephalon and the spinal cord were also labelled.

Overall, the progeny of the C1 blastomeres were restricted to mesodermal and endodermal derivatives (Figure 2b). Most of the cells derived from C1 blastomeres contributed to the anterior notochord. Mixing of labelled and unlabelled cells was evident in the posterior region of notochord. Truncal somites and caudal mesoderm contained C1 derived cells. The ventral part of the foregut also derived from blastomere C1.

Lateral blastomeres.The descendant cells of the B2 and B3 blastomeres localized in ectodermal and mesodermal derivatives. The progeny of these blastomeres were scattered both in the epidermis and in dorsal (B3) or ventral (B2) parts of the rhombencephalon. B2 and B3 derived cells were mainly found in the head mesenchyme at the periphery of neural and otic vesicles. Somitic cells and cardiac arches were also labelled.

Cells derived from C2 blastomeres contributed to head mesenchyme, somites, lateral plates and cardiac vesicles. Lateral parts of the foregut also derived from C2.

The progeny of the C3 blastomeres mainly formed lateral plates and pronephros. Few C3 derived cells were present in truncal epidermis, somites and archenteron.

Ventral blastomeres. Most of the descendant cells of the B4 blastomeres were found in the ventral and lateral epidermis of the cephalic and truncal regions (Figure 2c). At the tail-bud stage the C4 derived cells localize mainly in ventral and lateral epidermis, lateral plate and blood islets of the truncal and posterior parts of the embryo (Figure 2d).

The marking experiments presented here were focused on the 32-cell stage of selected embryos. Our results confirm and extend the pioneer observations done on urodele fate mapping ([20], [21]). The descendant cells of B and C blastomeres contribute to more than one primary germ layer. Labelled cells remained coherent throughout the cleavage period. Further due to morphogenetic movements, they were redistributed in different derivatives. These observations agree well with those done in *Xenopus* ([22], [23]). They point out that the slow mixing and rearrangement of cells within each primary germ layer are fundamental mechanisms with respect to the segregation of different cell lineages that occurs during early amphibian development.

Literature Cited

1. J. Newport and M. Kirschner, *Cell*, **30**, 1982, p. 675-686.
2. J. Signoret and J. Lefresne, *Ann. Embryol. Morph.*, **4**, 1971, p. 113-123.
3. T.M. Woolf, C. Jennings, M. Rebagliata and D.A. Melton, *Nucleic Acids Res.*, **18**, 1990, p. 1763-1769.
4. J.M. Dayle, J.A. Walder and D.L. Weeks, *Nucleic Acids Res.*, **18**, 1990, p. 4751-4757.
5. R.J. Detrick, D. Dickey and C.R. Kintner, *Neuron*, **4**, 1990, p. 493-506.
6. T. Fujimori, S. Miyatani and M. Takeichi, *Development*, **110**, 1990, p. 97-104.
7. K.W.Y. Cho, E.A. Morita, C.V.E. Wright and E.M. De Robertis, *Cell*, **65**, 1991, p. 55-64.
8. A.P. Mc Mahon and R.T. Moon, *Cell*, **58**, 1989, p. 1075-1084.
9. M. Mechali, G. Evan and M. Gusse, *C.R. Acad. Sci. Paris*, **308**, 1989, p. 213-218.
10. T. Darribère, K. Guida, H. Larjava, K.E. Johnson, K.M. Yamada, J.P. Thiery and J.C. Boucaut, *J. Cell Biol.*, **110**, 1990, p. 1813-1823.
11. J.M.W. Slack, *J. Embryol. Exp. Morph.*, **80**, 1984, p. 289-319.
12. L. Gallien and M. Durocher, *Bull. Biol. Fr. Belg.*, **91**, 1957, p. 97-114.
13. O.Nakamura and K. Kishiyama, *Proc. Japan Acad.*, **47**, 1971, p. 407-412.
14. R.L. Gimlich and J.C. Gerhart, *Dev. Biol.*, **104**, 1984, p. 117-130.
15. D.L. Shi, M. Delarue, T. Darribere, J.F. Riou and J.C. Boucaut, *Development*, **100**, 1987, p. 147-161.
16. L. Dale and J.M.W. Slack, *Development*,, **99**, 1987, p. 527-551.
17. H. Cleine and J.M.W. Slack, *J. Embryol. Exp. Morph.*, **86**, 1985, p. 247-269.
18. M. Delarue, S. Sanchez, K.E. Johnson, T. Darribere and J.C. Boucaut, *Development*, (sous presse)
19. S.A. Moody, *Dev. Biol.* **119**, 1987, p. 560-578.
20. W. Vogt, *Wilhelm Roux Arch. Entw. Mech. Org.*, **120**, 1929, p. 384-706.
21. J. Pasteels, *J. Exp. Zool.*, **89**, 1942, p. 255-281.
22. R. Wetts and S.E. Fraser, *Development*, **105**, 1989, p. 9-15.
23. P. Wilson and R. Keller, *Development*, **112**, 1991, p. 289-300.